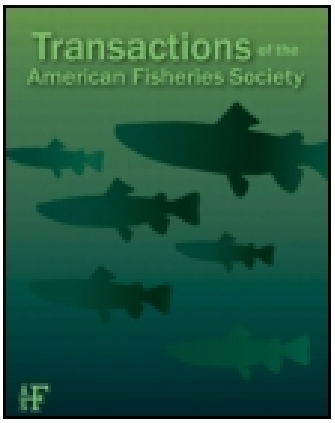

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Effects of High Tissue Concentrations of Selenium on Reproduction by Bluegills[1]

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Abstract.—Recent studies have associated high body concentrations of selenium with declines in fish populations inhabiting cooling reservoirs of coal-fired electric power plants. Because some evidence indicated that these declines resulted from reduced reproduction, we made a series of 18 artificial crosses of bluegills *Lepomis macrochirus* possessing high and low body concentrations of Se to determine whether elevated Se in parents reduced viability of gametes or increased mortality of embryos and larvae. Bluegills with high body concentrations of Se were obtained from Hyco Reservoir (cooling water source of a coal-fired power plant) and those with low body concentrations were obtained from nearby Roxboro City Lake, North Carolina. Neither percent fertilization nor percent hatch of eggs differed significantly among the parent combinations. However, all crosses (8) that included females with high Se body concentrations resulted in larvae with edema; such larvae did not survive to the swim-up stage. Only one of these crosses produced some normal larvae (35%). Mean Se concentrations in the gonads and carcass (body minus gonad) were more than 20 times higher in bluegills from Hyco Reservoir (average = 7.94 mg/kg) than in those from Roxboro City Lake (average = 0.38 mg/kg). The high Se concentrations in ovaries of Hyco Reservoir bluegills and in their progeny suggested that Se was transferred from females to offspring and caused edema in larvae. This abnormality resulted in mortality of affected larval bluegills—and consequently may have caused reductions in the bluegill populations of selenium-enriched reservoirs.

The combustion of fossil fuels, a major anthropogenic source of selenium in the environment, yields up to 11,022 t of Se per year (Adams and Johnson 1981). At some coal-fired power plants, fly ash with adsorbed Se (1.2 to 16 mg/kg) is collected by electrostatic precipitators and flushed to settling basins, from which soluble forms of Se enter the main reservoir. Selenium from the ash pond effluent results in high waterborne concentrations in cooling reservoirs and bioaccumulates in the biota inhabiting them (Cumbie 1980).

The median lethal concentrations (LC50) of selenite for several species of freshwater fish range from 0.62 to 28.5 mg/L in acute exposures and from 0.088 to 0.113 mg/L in chronic exposures (USEPA 1980). Although these concentrations of inorganic Se are high in relation to those in natural systems, various studies have demonstrated the importance of bioaccumulation and uptake of the more available organic forms (Sandholm et al. 1973; Chau et al. 1976). The accumulation of Se by fish in cooling reservoir water was documented by Cumbie (1978). In situ 28-week exposures of golden shiners *Notemigonus crysoleucas* showed a significantly higher accumulation of Se in fish held closer to ash pond effluents than in those held farther from ash pond effluents (CPL 1984a).

Recent declines in the fish populations of several cooling reservoirs of coal-fired electric power plants have been accompanied by increasing tissue concentrations of Se. Estimates of adult fish densities (number per hectare) in eight selected coves decreased from about 7,200 in 1973 to 840 in 1976 in Belews Lake, North Carolina (Cumbie and Van Horn 1979), and by 38–75% from 1979 to 1980 in four coves of Hyco Reservoir, North Carolina (CPL 1981a). Even more striking were the decreases in estimated young-of-year densities (four species) from 2,300 to 2 per hectare in eight coves of Belews Lake and larva densities that decreased from 1,060/1,000 m^3 to 15/1,000 m^3 along three transects in Hyco Reservoir during the same period. Cumbie (1978) attributed the decline in fish populations in Belews Lake to decreased reproduction resulting from high body concentrations of Se. The mean Se concentration in skeletal mus-

[1] Based in part on a doctoral dissertation by R. B. Gillespie in the Department of Zoology, The Ohio State University, Columbus, Ohio.

cle of bluegills *Lepomis macrochirus* was 11.1 mg/kg wet weight in fish collected from Belews Lake in 1976 and 1977 (Cumbie and Van Horn 1979) and 6.7 to 9.7 mg/kg in fish from Hyco Reservoir in 1977 to 1980 (CPL 1981b). In contrast, the mean whole-body concentration of Se was only 0.44 mg/kg (wet weight) in fish collected for the National Pesticide Monitoring Program (NPMP) at 109 stations throughout the United States in 1977 and 1978 (Baumann and May 1982).

Earlier research had indicated that average waterborne concentrations of Se (9 to 12 μg/L) in Hyco Reservoir (CPL 1981b) were too low to cause acute or chronic mortality to either adults or larvae. We proposed and tested two alternative hypotheses to explain the seemingly poor reproduction by these fish: decreased gamete viability or increased mortality of embryos and larvae from parentally transferred selenium.

Methods

Adult bluegills were captured by electrofishing and with fyke nets in Hyco Reservoir, a 2,000-hectare impoundment that is a source of cooling water for the Roxboro Steam Generating Station near Roxboro, North Carolina. Adult bluegills were also collected in Roxboro City Lake, a nearby impoundment used as a municipal water supply, which has a fish community with low Se body burdens. Bluegills were chosen as the study species because they could be easily caught in both reservoirs and bluegill population declines have been documented in selenium-enriched reservoirs.

All possible combinations of bluegill parents from Hyco Reservoir and Roxboro City Lake were artificially crossed in June and July, 1982 and 1983. To estimate fertilization success, we stripped three subsamples of 100 to 500 eggs per female and combined them with about 2 mL of sperm in glass Syracuse dishes. Zygotes were reared in Roxboro City Lake water and percent fertilization was estimated as the proportion of mitotically active zygotes 2–3 h after fertilization. To estimate hatching success, we combined gametes as described above and transferred three subsamples of 100–300 embryos per cross to egg cups and maintained them in closed aquaria with circulating Roxboro City Lake water. Hatching occurred at about 2 d after fertilization at temperatures of 22–25°C. Once hatching began, dead embryos were removed and percent hatch was based on the number of yolk-sac larvae. Some crosses were used for estimates of both percent fertilization and percent hatch. Others were used only for fertilization or hatching estimates.

In 1982, about 200 embryos from eight crosses were preserved at intervals up to 40 h after fertilization, and about 450 larvae were preserved at intervals of 40–180 h after fertilization. In 1983, about 1,800 larvae from 10 crosses were preserved at intervals of 40–150 h after fertilization for Hyco female crosses and 40–300 h after fertilization for Roxboro female crosses.

The following samples of adult bluegills were collected from Hyco Reservoir and frozen for Se analysis: in 1981, six fish of each sex; in 1982 and 1983, 32 males and 28 females. In 1982 and 1983, 17 male and 19 female bluegills were collected from Roxboro City Lake and frozen for Se analysis. All gonads were dissected from the carcass and some of these were analyzed for Se.

Selenium residue analyses for adult bluegills were performed at the Columbia National Fisheries Research Laboratory (CNFRL) by a dry-ash technique described by May (1982). The six bluegills of each sex collected in 1981 from Hyco Reservoir and four females collected in 1982 from Hyco Reservoir and Roxboro City Lake (two per lake) were also analyzed for six other trace elements (As, Cd, Cu, Hg, Pb, Zn) at CNFRL (Lowe et al. 1985). Analysis for trace elements of fishes from all sites (Hyco, Roxboro, and NPMP) were performed at CNFRL. Larvae from two crosses each of Hyco Reservoir parents and Roxboro City Lake parents (in 1983) were frozen and analyzed for total Se at the Research Reactor Facility of the University of Missouri, Research Park, Columbia, Missouri. Larvae from each cross were grouped by parent combination and analyzed as composite samples.

Percent fertilization and percent hatch were transformed to arcsines prior to statistical analyses. One-way analysis of variance was used to estimate significant differences for mean percent fertilization and percent hatch among crosses and paired Student's t-tests were used to determine significant differences in mean Se concentrations among carcasses and gonads of bluegills from Hyco Reservoir.

Results

Among 18 crosses made during the 2 years, no significant differences were found in percent fertilization or in percent hatch among parent combinations (analysis of variance; $P > 0.05$; Table 1). In contrast, larvae collected from crosses involving Hyco Reservoir females had a gross abnormal morphology, characterized by general ede-

TABLE 1.—Mean percent fertilization and percent hatch for artificial crosses of bluegills from Hyco Reservoir (H) and Roxboro City Lake (R), June and July 1982 and 1983. Percentages were transformed to arcsines prior to statistical analysis.

Cross (male × female)	Fertilization			Hatch		
	Number of		Percent fertile	Number of		Percent hatch
	Crosses	Replicates	Mean±SD	Crosses	Replicates	Mean±SD
R × H	5	13	73±7	5	12	75±12
H × H	7	20	69±10	5	16	71±11
R × R	4	10	78±13	4	10	66±15
H × R	2	6	69±19	3	9	73±15

ma (Figure 1), that became evident as early as 100 h after fertilization. Larvae having the edema were less motile than other larvae, did not swim normally, and died before they reached the swim-up stage. Only larvae from crosses involving a Hyco female were abnormal. Of eight crosses using Hyco females, only one produced some (35%) seemingly normal larvae (Table 2); all of the rest showed the edema described.

Concentrations of Se in carcasses of bluegills from Hyco Reservoir were 16–21 times higher than those in bluegills from Roxboro City Lake during 1982 and 1983 (Table 3). In bluegills from both reservoirs, average Se concentrations were higher in the ovaries than in the carcass. Selenium concentrations in the testes, however, showed no consistent relationship to carcass values.

Because the composition and volume of yolk changes in ovaries during maturation, the concentration of Se in ovaries may also change during the maturation process. Therefore, female bluegills from Hyco Reservoir were divided into two groups, those stripped of eggs for crosses and those not stripped; mean Se concentrations were then calculated separately for carcasses and ovaries within each group (Table 4). The mean concentrations of Se were similar in ovaries and carcasses of bluegills whose ova were removed for crosses, whereas mean ovarian Se was significantly greater than carcass Se in bluegills whose ova were not stripped.

Among bluegill larvae collected from artificial crosses, Se concentrations were nearly 10 times higher in larvae from Hyco Reservoir parents than in those from Roxboro City Lake parents (Table 5).

Trace element scans of bluegills collected in 1981 and 1982 showed that, of seven potentially toxic elements tested, only Se in Hyco Reservoir fish was dramatically greater than the averages for samples collected for the National Pesticide Monitoring Program (Table 6).

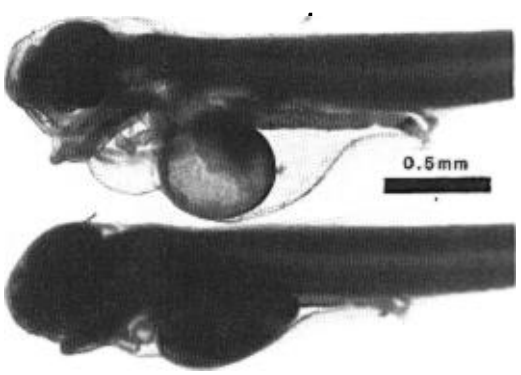

FIGURE 1.—Edematous bluegill larva (top) from Hyco Reservoir parents at 143 h postfertilization and normal bluegill larva (bottom) from a Roxboro City Lake female × Hyco male cross at 139 h postfertilization.

Discussion

In the 1950s, Se was suspected of causing mortalities of fish stocked in Sweitzer Lake, Colorado,

TABLE 2.—Incidence of edema in bluegill larvae subsampled from artificial crosses of Hyco Reservoir females during 1982 and 1983. Selenium concentrations in corresponding ovaries of parents are also shown.

Year and number of larvae examined[a]	Percent abnormal	Ovarian Se[b] (mg/kg wet weight)
1982		
40	100	6.68
46	100	6.78
46[c]	100	6.68
54[c]	100	6.78
1983		
63	100	8.00
74	65	5.79
49	100	7.42
51[c]	100	7.21

[a] Only larvae older than 100 h after fertilization are included.
[b] Ovaries with identical Se concentrations represent a single female whose eggs were used for two crosses with different males.
[c] Crosses with males from Roxboro City Lake; other crosses were with Hyco Reservoir males.

TABLE 3.—Mean selenium concentrations in carcasses and gonads (mg/kg wet weight) of bluegills collected from Hyco Reservoir and Roxboro City Lake in spring and summer 1982 and 1983. Gonads from some fish were grouped for composite analysis. Selenium concentrations reported include fish from cross experiments and others not used for cross experiments.

Sex and tissue	Hyco Reservoir		Roxboro City Lake	
	Number of samples	Mean Se±SD (mg/kg)	Number of samples	Mean Se±SD (mg/kg)
Males				
Carcass	32	7.81±2.66	17	0.37±0.05
Testis	23	4.37±1.57	12	0.50±0.10
Females				
Carcass	28	5.91±2.53	19	0.37±0.09
Ovary	22	6.96±2.80	14	0.66±0.13

TABLE 5.—Total selenium concentrations in bluegill larvae and parent ovaries from artificial crosses of parents from Roxboro City Lake (R × R) and Hyco Reservoir (H × H). Larvae from each parent type, hatched and reared in Roxboro City Lake water, were analyzed as one composite sample.

Cross	Ovarian Se[a] (mg/kg dry weight)	Larvae	
		N	Se (mg/kg dry weight)
R × R			
1	2.95	18	3.10
2	3.62	291	
H × H			
1	54.16	71	28.20
2	38.79	222	

[a] Conversions of ovarian selenium concentrations were based on an assumed 85% moisture content. Ovaries were analyzed for Se after eggs were stripped for crosses.

where tissue concentrations up to 8 mg/kg occurred in fish from water that averaged 9.4 μg/L Se (Barnhart 1957). Reproductive failure in fish populations with high Se body concentrations (mean = 14.3 mg/kg) was suggested as the cause of drastic declines in fish densities and the eventual elimination of centrarchids, including largemouth bass *Micropterus salmoides* and bluegills, in Belews Lake, North Carolina (Cumbie 1978; Cumbie and Van Horn 1979). A decline in abundances of adults and larvae in Hyco Reservoir was associated with high adult body concentrations of Se, averaging 6.7 to 9.7 mg/kg (CPL 1981b).

Earlier bioassay studies indicated, however, that average concentrations of Se in water of Hyco Reservoir (9 to 12 μg/L) would be below that necessary to cause significant mortality in eggs and larval fishes. Huckabee and Griffith (1974) and Niimi and LaHam (1975) found that concentrations below 3 and 5 mg/L Se did not affect embryo hatchability of common carp *Cyprinus carpio* or embryo mortality of zebra danio *Danio* [*Brachydanio*] *rerio*. Even the more sensitive egg and larval stages of rainbow trout *Salmo gairdneri* did not experience increased mortality or developmental abnormalities at concentrations less than 30 μg/L (Goettl and Davies 1978; Hodson et al. 1980).

All of these earlier data are compatible with our findings that larva abnormalities may have been caused by Se transferred to the egg from the female parent. All female bluegills from Hyco Reservoir produced at least some abnormal larvae; Se concentrations in ovaries were all above 5 mg/kg. Edema developed even when eggs were fertilized by males with low Se body concentrations and were held in Roxboro City Lake water. Furthermore, if the relationship between Se in ovaries and carcasses of bluegills not stripped of eggs were the

TABLE 4.—Mean selenium concentrations (mg/kg wet weight) in carcasses and ovaries of bluegills collected form Hyco Reservoir during spring and summer 1982 and 1983. Stripped = fish previously stripped of gametes for use in artificial cross experiments. Unstripped = fish not used for cross experiments.

Sex and tissue	Stripped		Unstripped	
	N	Se±SD (mg/kg)	N	Se±SD (mg/kg)
Females				
Carcass	7	7.25±1.90	14	4.06±1.83
Ovary	7	7.13±0.79	14	6.66±3.39[a]

[a] Significantly different from corresponding carcass concentration (*t*-test; $P < 0.05$).

TABLE 6.—Mean trace element concentrations (mg/kg wet weight) for bluegill carcasses from Hyco Reservoir (H), Roxboro City Lake (R), and the U.S. national averages of whole-body concentrations reported by the National Pesticide Monitoring Program (NPMP).

Source	Se	As	Cu	Zn	Hg	Pb	Cd
H[a]	7.20	0.11	0.99	28.8	0.12	0.26	0.01
H[b]	6.90	0.05	0.36	25.9	0.01	0.05	0.007
R[c]	0.37	0.05	0.30	25.3	0.09	0.05	0.005
NPMP[d]	0.48	0.15	0.66	22.0	0.12	0.17	0.03

[a] $N = 12$ for Se (1981); other trace elements are based on single composite of 12 fish.

[b] $N = 60$ for Se (1982–1983); $N = 2$ (females) for other trace elements.

[c] $N = 36$ for Se (1982–1983); $N = 2$ (females) for other trace elements.

[d] Averages for 1980 and 1981 are from 103 sampling stations.

same for bluegills whose ova were stripped, then Se concentrations in ovaries of bluegills used for crosses would have been greater before removal of mature eggs. High Se concentrations in larvae produced by such females, even when the larvae were reared in low-Se water, also supports the hypothesis of parentally transferred Se to developing larva.

In bioassay studies conducted by Carolina Power and Light Company (CPL 1984b, 1984c) bluegill embryos were exposed to various dilutions of effluent from ash ponds adjacent to Hyco Reservoir. In these experiments, Hyco Reservoir females produced edematous larvae regardless of water type, whereas larvae hatched from eggs of Roxboro City Lake females showed no edema, even after exposure to ash pond effluent. These results support our conclusion that Se is transferred from the female to eggs and larvae. This mechanism of parental transfer to Se probably occurs in the feral population of bluegills in Hyco Reservoir, as well as in other selenium-enriched impoundments.

Other potentially toxic trace elements in carcasses of bluegills from Hyco Reservoir that might have caused teratogenic effects were either below or only slightly above reference lake values and U.S. national averages of whole-body concentrations established by the National Pesticide Monitoring Program. On the other hand, the mean concentrations of Se in bluegills from Hyco Reservoir collected in both years were roughly 10 times higher than the mean nationwide Se residue concentrations found in the NPMP in 1980–1981. Thus Se appears to be the teratogenic agent causing the edema in larvae and population declines in this reservoir.

The current U.S. Environmental Protection Agency water quality criterion for selenium to protect freshwater life is 35 μg/L (USEPA 1980), and some workers have stated that concentrations as high as 52 μg/L may not endanger aquatic species (Adams and Johnson 1981). The parent fish that produced 100% edematous larvae in the present study were taken from a lake in which average yearly waterborne Se concentrations (several years preceding 1981) were reported to be 10 μg/L (CPL 1981a). At this time we do not know the exposure concentrations of Se to bluegills, the chemical species of Se in the water of Hyco Reservoir, or the species of Se in carcass, ovary, egg, or larva. However, we agree with the conclusions of Lemly (1982) and Baumann and May (1982) that the existing criterion for Se should be reviewed in light of potential biaccumulation and teratogenic effects.

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